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16. ABSTRACT

Lipase preparations were treated *in vitro* with 100 chemicals of various classes, many of which are environmental pollutants, to determine their effect upon enzyme activity. The greatest inhibition was caused by mercuric ion and certain heavy metal cations; almost as inhibiting were pentachlorophenol, dicofol, 2,4,5-trichlorophenol, and DDT. Less inhibition was found with other inorganic salts, organo-metals, other organic chemicals, and some pesticides. Apart from dichromate ion, the anions studied had a low to negligible effect on lipase. A mixture of 10 inorganic salts had an approximately cumulative inhibiton effect on enzyme activity. None of the chemicals studied caused a measurable activation of the enzyme.

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Effect of Water Pollutants and Other Chemicals Upon the Activity of Lipase *in vitro*

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Abstract. Lipase preparations were treated *in vitro* with 100 chemicals of various classes, many of which are environmental pollutants, to determine their effect upon enzyme activity. The greatest inhibition was caused by mercuric ion and certain heavy metal cations; almost as inhibiting were pentachlorophenol, dicofol, 2,4,5-trichlorophenol, and DDT. Less inhibition was found with other inorganic salts, organo-metals, other organic chemicals, and some pesticides. Apart from dichromate ion, the anions studied had a low to negligible effect on lipase. A mixture of 10 inorganic salts had an approximately cumulative inhibitory effect on enzyme activity. None of the chemicals studied caused a measurable activation of the enzyme.

Attention was focused upon an important lipid enzyme which might be particularly vulnerable to some highly lipid-soluble environmental chemicals that bioaccumulate in lipid-rich tissue when their rate of degradation or elimination is relatively slow.

Little information is available concerning the effect of environmental pollutants upon the activity of lipase (E.C. 3.1.1.3, glycerol hydrolase ester). The effect of protamine as an inhibitor of lipase has been reported (Korn 1955), as has the effect of arsenicals (Singer and Barron 1945), di-isopropyl-*p*-nitro phenyl phosphate (Myers 1956), certain anionic detergents (Wills 1955), and the biocides, aldrin, sevin, lindane, 2,4-D, heptachlor, and DDT (Guilbault and Sadar 1969). Zinc, copper, mercury, iodine, *p*-chloro-mercuribenzoate, and versene also affect this enzyme (Wills 1960).

One hundred organic and inorganic chemicals were tested *in vitro* for their effect upon lipase activity (Table 1). They are representative of some of the major types of chemical water pollutants, but certain other compounds were also included. Since ecosystem pollution usually involves mixtures of chemicals, not only was the effect of single agents upon enzyme activity studied, but also the effect of a mixture composed of Hg(II), Pt(IV), Ni(II), Cd(II), Cu(II), Cr(III), Pd(IV), Zn(II), Fe(III), and Au(III).

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Table 1. Chemicals tested for their effect on lipase activity (A = antibiotic, F = fungicide, FM = fumigant, H = herbicide, I = insecticide, and R = rodenticide)

A.	Thiomides	
	Captan (F)	N-(Trichloromethylthio)-4-cyclohexene-1,2-dicarboximide
B.	Thiocarbamates	
	Thiram (F)	Tetramethylthioperoxydicarbonic diamide
	Eptam® (F)	*S*-ethyl dipropylthiocarbamate
	Ferbam (F)	tris(dimethylcarbamodithioato-*S*,*S*')iron
C.	Carbamates	
	IPC® (H)	Isopropyl-N-phenyl carbamate
	Carbaryl (I)	Sevin®; 1-Naphthyl-N-methyl carbamate
D.	Organothiophosphates	
	Malathion (I)	diethyl[(dimethoxyphosphinothioyl)thio]-butanedioate
	Diazinon (I)	*O*,*O*-diethyl *O*-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl]phosphorothioate
	Methyl parathion (I)	*O*,*O*-dimethyl *O*-(4-nitrophenyl)-phosphorothioate
E.	Urea derivatives	
	Monuron (H)	3-(*p*-Chlorophenyl)-1,1-dimethyl urea
	Bromacil (H)	5-bromo-3-*sec*-butyl-6-methyluracil
F.	Antibiotics and other biocides	
	Warfarin (R)	3[α-Acetonylbenzyl]-4-hydroxycoumarin
	Penicillin (A)	
	Neomycin (A)	
	Acetazolamide (A)	Diamox; N-(5-Sulfamoyl-1,3,4-thiadiazol-2-yl) acetamide
	Puromycin	
G.	Aromatic halogenated hydrocarbons	
	DDT (I)	Dichlorodiphenyltrichloroethane (mix of *o*,*p* and *p*,*p*')
	Dicofol (I)	Kelthane®; 4-chloro-α-(4-chlorophenyl)-α-(trichloromethyl)benzenemethanol
	Chlorobenzene	
	p-Dichlorobenzene (FM)	
	Hexachlorobenzene	
H.	Aliphatic halogenated hydrocarbons	
	Lindane (I)	(1α,2α,3β,4α,5α,6β)-1,2,3,4,5,6-Hexachlorocyclohexane (γ isomer)
	Dieldrin (I)	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-*endo*-1,4-*exo*-5,8-dimethanonaphthalene
	Chloroform	
	Carbon tetrachloride	
	1,2-Dichloroethane	
	1,2-Dichloropropane	
	1,1,1-Trichloroethane	
	Trichloroacetic acid	
I.	Phenols, halogenated phenols and halophenoxy acids	
	Phenol (A)	
	2,4,5-Trichlorophenol (F)	
	Pentachlorophenol (F)	
	2,4-D (H)	2,4-Dichlorophenoxy acetic acid
	2,4,5-T (H)	2,4,5-Trichlorophenoxy acetic acid
	Resorcinol	
	α-Napthol	

Table 1. (Continued)

J. Solvents and other industrial chemicals
- Aniline
- Acetone
- Methanol (A)
- Ethanol (A)
- Sodium dodecyl sulfate (A)
- Benzene
- 4,6-Dinitro-o-cresol (H)

K. Inorganic salts (active cation)
- $AgNO_3$
- $AlCl_3$
- $BaCl_2 \cdot 2H_2O$
- $BiCl_3$
- $CaCl_2$
- $CdCl_2$
- $CoCl_2 \cdot 6H_2O$
- $CrCl_3$
- $CuCl_2 \cdot 2H_2O$
- $FeCl_2$
- $FeCl_3 \cdot 6H_2O$
- $HAuCl_4 \cdot 3H_2O$
- $HgCl_2$
- K_2PtCl_6
- K_2PdCl_4
- LiCl
- $MgCl_2$
- $MnCl_2$
- $NiCl_2 \cdot 6H_2O$
- NaCl
- Na_2PdCl_4
- $Pb(NO_3)_2$
- $SnCl_2 \cdot 2H_2O$
- $SnCl_4 \cdot 5H_2O$
- $ZnCl_2$

L. Inorganic salts (active anion)
- NaCN
- NaF
- NaBr
- NaI
- NaN_3
- $Na_2S \cdot 9H_2O$
- $NaIO_4$
- $NaBH_4$
- $K_2Cr_2O_7$
- Na_2HAsO_4
- $NaAsO_2$
- $Na_2TeO_4 \cdot 2H_2O$
- Na_2SeO_4
- Na_2SeO_3
- $NaNO_2$
- $NaNO_3$

M. Organo-metals
- Methylmercuric chloride
- Phenylmercuric chloride

Table 1. (Continued)

N.	Carcinogens	
	DEB	Diepoxybutane
	MMS	Methyl methane sulfonate
	DMCC	Dimethyl carbamoyl chloride
	CMEE	*bis*-Chloromethyl ethyl ether
O.	Miscellaneous chemicals	
	Iodoacetic acid	
	Piperonyl butoxide	3,4-Methylenedioxy-6-propylbenzyl-*n*-butyl diethylene glycol ether
	$EDTA \cdot 2Na \cdot 2H_2O$	Ethylenediamine tetraacetic acid
	NAD	Nicotinamide adenine dinucleotide (oxidized)
	NADH	Nicotinamide adenine dinucleotide (reduced)
	Lipoic acid	
	Glutathione	
	Ascorbic acid	
	ATP	Adenosine-5'-tri-phosphate
	Adrenaline	

Materials and Methods

Following an established fluorometric procedure (Jacks and Kirchner 1967, Guilbault and Sadar 1969), the enzymatic reaction was carried out by mixing 0.900 ml of phosphate buffer (0.10 M, pH 7.0) with 1.250 ml of either buffer (blank) or the test reagent (inhibitor chemical) in buffer, and then adding 0.300 ml of the enzyme preparation (20 mg/15 ml buffer).[1] After preincubation for 15 min, the substrate was added (50μl of 3 mg/6 ml methanol), rapidly mixed, and immediately analyzed fluorometrically at 320/450 nm. The substrate was prepared from 4-methyl umbelliferone and heptanoyl chloride (Jacks and Kirchner 1967). A strip chart recording provided a record of the rate of generation of the fluorescent product. The reaction-rate limits (slopes) were determined from enzyme preparations with no inhibitors (100% activity) and from enzyme blank preparations (0% activity), and the relative degree of activity for the test chemicals was determined with reference to these limit values. The specific activity was determined from a standard curve prepared from 4-methyl umbelliferone (1 to 100μM). All analyses at all test concentrations were carried out in triplicate, and mean values were determined. For each test chemical, serial dilutions were made from 0.01 M stock solutions (or suspensions) until no effect on enzyme activity was detectable. However, for NaCl, which was used as a reference for the least toxic chemical studied, solutions up to 5 M were analyzed. For each of these chemicals, graphs were prepared showing percentage activity vs. concentration, from which values for I_{50} and I_1 (molarity of chemical causing 50% and 1% inhibition of enzyme activity) were obtained.

In addition to the tests with individual chemicals, one mixture was studied composed of $HgCl_2$, K_2PtCl_4, $NiCl_2 \cdot 6H_2O$, $CdCl_2$, $CuCl_2 \cdot 2H_2O$, $CrCl_3$, K_2PdCl_4, $ZnCl_2$, $FeCl_3 \cdot 6H_2O$, and $HAuCl_4 \cdot 3H_2O$. The amount of each component in the stock preparation was based upon that concentration which alone constituted a threshold ("effect-no-effect") value. Dilutions were then made of this solution and enzyme analyses carried out as with the single toxicant samples.

Results and Discussion

The specific activity of the enzyme preparation was 3μM/mg protein per min. The 100 chemicals analyzed have been arranged in Table 2 according to their

[1] Lipase, E.C.3.1.1.3. type II, hog pancreas, Sigma Chemical Co., P.O. 14508, St. Louis, MO 63178.

Table 2. Molar concentration of test reagent causing a 50% inhibition (I_{50}) and a threshold effect (I_t) on lipase activity and showing an effect level relative to NaCl (I_t NaCl/I_t test chemical)

Chemical (active component)	I_{50}	I_t	I_t NaCl / I_t test chemical	Chemical (active component)	I_{50}	I_t	I_t NaCl / I_t test chemical
Hg(II)	3×10^{-5}	3×10^{-6}	6.7×10^{5}	Carbaryl	9×10^{-3}	8×10^{-5}	2.5×10^{4}
Pb(IV)	4×10^{-5}	2×10^{-6}	1.0×10^{6}	Dimethyl carbamyl chloride	1×10^{-2}	7×10^{-4}	2.9×10^{3}
Ag(I)	7×10^{-5}	1×10^{-6}	2.0×10^{6}	NAD	2×10^{-2}	1×10^{-3}	2.0×10^{3}
Pd(II)	1×10^{-4}	6×10^{-5}	3.3×10^{4}	Phenol	4×10^{-2}	7×10^{-3}	2.9×10^{3}
Pentachlorophenol	1×10^{-4}	8×10^{-6}	2.5×10^{5}	Co(II)	4×10^{-2}	1×10^{-2}	2.0×10^{3}
Dicofol	2×10^{-4}	5×10^{-6}	4.0×10^{5}	Sn(II)	5×10^{-2}	1×10^{-2}	2.0×10^{3}
2,4,5-Trichlorophenol	3×10^{-4}	3×10^{-6}	6.6×10^{5}	Zn(II)	5×10^{-2}	2×10^{-4}	1.0×10^{4}
DDT	3×10^{-4}	2×10^{-5}	1.0×10^{5}	Aniline	5×10^{-2}	7×10^{-3}	2.9×10^{3}
Warfarin	3×10^{-4}	1×10^{-5}	2.0×10^{5}	Methyl Hg(I)	5×10^{-2}	7×10^{-4}	2.9×10^{3}
4,6-Dinitro-o-cresol	4×10^{-4}	1×10^{-5}	2.0×10^{5}	Methyl methane sulfonate	5×10^{-2}	1×10^{-2}	2.0×10^{3}
$Cr_2O_7^{-2}$	4×10^{-4}	6×10^{-5}	3.3×10^{4}	Cr(III)	5×10^{-2}	7×10^{-4}	2.9×10^{3}
Malathion	5×10^{-4}	7×10^{-5}	2.9×10^{4}	Diazinon	[a]	1×10^{-5}	2.0×10^{5}
Methyl parathion	5×10^{-4}	7×10^{-5}	2.9×10^{4}	Ferbam	[a]	5×10^{-5}	4.0×10^{4}
Au(III)	5×10^{-4}	4×10^{-5}	5.0×10^{4}	*p*-Dichlorobenzene	[a]	1×10^{-4}	2.0×10^{4}
Pt(IV)	5×10^{-4}	5×10^{-5}	4.0×10^{4}	Epsam	[a]	2×10^{-4}	1.0×10^{4}
bis-Chloromethyl ethyl ether	6×10^{-4}	8×10^{-5}	2.5×10^{4}	Adrenaline	[a]	1×10^{-2}	2.0×10^{3}
NADH	7×10^{-4}	8×10^{-5}	2.5×10^{4}	ATP	[a]	1×10^{-2}	2.0×10^{3}
Fe(III)	1×10^{-3}	5×10^{-5}	4.0×10^{4}	F^{-1}	[a]	1×10^{-2}	2.0×10^{3}
Cu(II)	2×10^{-3}	1×10^{-4}	2.0×10^{4}	Acetazolamide	[a]	1×10^{-2}	2.0×10^{3}
Pb(II)	2×10^{-3}	8×10^{-5}	2.5×10^{4}	IO_4^{-1}	[a]	1×10^{-2}	2.0×10^{3}
Piperonyl butoxide	2×10^{-3}	5×10^{-4}	4.0×10^{3}	Cd(II)	[a]	1×10^{-2}	2.0×10^{3}
Phenyl Hg(I)	2×10^{-3}	3×10^{-5}	6.7×10^{4}	Al(III)	[a]	2×10^{-2}	1.0×10^{3}
Thiram	2×10^{-3}	3×10^{-5}	6.7×10^{4}	Li(I)	[a]	2×10^{-2}	1.0×10^{3}
Dodecyl sulfate	2×10^{-3}	5×10^{-4}	4.0×10^{3}	Acetone	[a]	2×10^{-2}	1.0×10^{3}
Lindane	3×10^{-3}	5×10^{-5}	4.0×10^{4}	Ni(II)	[a]	3×10^{-2}	6.7×10^{3}
Ethanol	3×10^{-3}	5×10^{-4}	4.0×10^{3}	IPC	[a]	5×10^{-2}	4.0×10^{3}
Sn(IV)	3×10^{-3}	8×10^{-4}	2.5×10^{3}	Puromycin	[a]	5×10^{-2}	4.0×10^{3}
Fe(II)	3×10^{-3}	2×10^{-3}	1.0×10^{3}	Iodoacetic acid	[a]	6×10^{-2}	3.3×10^{3}
Captan	3×10^{-3}	2×10^{-3}	1.0×10^{3}	Diepoxybutane	[a]	1×10^{-2}	2.0×10^{3}
Lipoic acid	4×10^{-3}	5×10^{-4}	4.0×10^{3}	NaCl	[a]	2	1
Dieldrin	7×10^{-3}	7×10^{-4}	2.9×10^{3}	b			

[a] $I_{50} > 5 \times 10^{-2}$ M (I_{50} not determinable)

[b] Negligible effect at 10^{-2} M: Ba(II), Ca(II), Mg(II), Mn(II), Bi(III), N_3^{-1}, CN^{-1}, S^{-2}, Br^{-1}, I^{-1}, TeO_4^{-2}, SeO_4^{-2}, SeO_3^{-2}, AsO_3^{-1}, AsO_4^{-3}, NO_3^{-1}, NO_2^{-1}, BH_4^{-1}, EDTA, neomycin, penicillin, glutathione, ascorbic acid, methanol, trichloroacetic acid, monuron, bromacil, $CHCl_3$, CCl_4, chlorobenzene, 1,2-dichloroethane, 1,2-dichloropropane, 1,1,1-trichloroethane, hexachlorobenzene, 2,4-D, 2,4,5-T, *o*-naphthol, resorcinol and benzene.

relative inhibitory effect upon lipase. The I_{50} values show that Hg(II) had the strongest inhibitory effect, followed very closely by Pd(IV). The chemicals Ag(I), Pd(II), pentachlorophenol, and dicofol were also highly inhibiting; only slightly less so were 2,4,5-trichlorophenol, DDT, and warfarin. With the exception of Cd(II), the enzyme generally showed a relatively high sensitivity to many heavy metal cations that are toxic to aquatic animals. Of the organic chemicals studied, the chlorinated phenols (pentachlorophenol and 2,4,5-trichlorophenol) and the chlorobenzene pesticides (dicofol and DDT) were the most inhibiting; warfarin (aromatic-oxygenated ketone) and the organophosphate pesticide malathion were only slightly less inhibiting. Other pesticides analyzed were at least 10 times less active than kelthane on a molar basis. The heterocyclic organo-thio phosphate diazinon elicited a slight effect at a relatively high concentration (0.01 M), even though it is structurally related to malathion. The organo-metals, phenyl mercuric chloride and methyl mercuric chloride, had an intermediate inhibitory effect. Although the oxidizing agent $Cr_2O_7^{-2}$ was quite inhibiting, the reaction with IO_4^{-1} was only slight. Some of the anions usually considered fairly toxic (i.e., F^{-1}, CN^{-1}, N_3^{-1}, I^{-1}) had only a slight or negligible effect. Of the four organic carcinogens studied, *bis*-chloromethyl ethyl ether was the most inhibiting, but 30 times less inhibiting than Hg(II).

The chlorinated phenols were significantly more inhibiting than phenol and the benzene chlorides. The chloroethanes, chloropropanes, and chlorophenoxy acetic acid chemicals had no effect. The chemical NADH was about 30 times more reactive than NAD^+; the reducing agent BH_4^{-1} had no effect. The common organic solvents ethanol, methanol, and acetone had a detrimental effect at concentrations of about 10^{-2} M. Acetazolamide, a strong inhibitor of carbonic anhydrase, had only a slight inhibiting effect. Carbaryl (sevin), a strong inhibitor of acetylcholinesterase, was only moderately inhibiting of lipase activity. Malathion, however, appears to cause about the same degree of inhibition in lipase as it does in cholinesterase (Olson and Christensen 1979). Kelthane and DDT, which significantly inhibit adenosine triphosphatase activity *in vitro* (Riedel and Christensen 1979), also inhibit lipase to approximately the same degree. Since lipase is quite strongly affected *in vitro* by some of the toxic halogenated hydrocarbons that are highly lipid soluble, it is possible that *in vivo* inhibition also occurs from these chemicals.

Sodium chloride is shown at the bottom of Table 2 and was analyzed to provide perspective. It was the least toxic and least inhibiting of all chemicals analyzed, showing a threshold effect (I_t) at a concentration of 2 M. The I_t values of all of the test agents were compared to that for NaCl and are shown as quotients in Table 2. Thus, for example, $AgNO_3$ is approximately 2,000,000 times more inhibiting than NaCl on a molar basis.

The experiment with the mixture of the 10 inorganic salts showed an inhibitory effect upon lipase that developed through an accumulation of effects from the components. The initial preparation based upon the threshold concentration for each component caused a 20% loss of activity. Full enzyme activity was found after the mixture preparation was diluted 10-fold. This information indicates that some toxicants, perhaps those with similar chemical and physical properties, have an accumulative ("additive") effect when they together are in contact with an enzyme. The interaction of toxicants, which may cause in-

creases or decreases in total effect upon biochemical and biological activity is an area of environmental study that needs much further work.

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